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R. Crescenzo, F. Bianco, P. Coppola, A. Mazzoli, G. Liverini,  
S. Iossa**

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## **Caloric Restriction Followed by High Fat Feeding Predisposes to Oxidative Stress in Skeletal Muscle Mitochondria**

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# Caloric Restriction Followed by High Fat Feeding Predisposes to Oxidative Stress in Skeletal Muscle Mitochondria

## Authors

R. Crescenzo, F. Bianco, P. Coppola, A. Mazzoli, G. Liverini, S. Iossa

## Affiliation

Department of Biology, University of Naples, Naples, Italy

## Key words

- caloric restriction
- mitochondria
- high fat diet

## Abstract

The purpose of the present study was to assess the impact of previous period of caloric restriction on energy balance and skeletal muscle mitochondrial energetics in response to high-fat (HF) diet. To this end, 1 group of rats was subjected to 2 weeks of caloric restriction with nonpurified diet and then fed HF diet (430 kJ metabolizable energy/day) for 1 week, while the second group was fed ad libitum with nonpurified diet for 2 weeks and then fed HF diet (430 kJ metabolizable energy/day) for 1 week. Body composition, energy balance, and glucose homeostasis were measured. Mitochondrial mass, oxidative capacity and efficiency, parameters of oxidative stress, and antioxidant defense were evaluated in subsarcolemmal and intermyofibrillar mitochondria

from skeletal muscle. Body energy and lipid content, plasma insulin, and metabolic efficiency were significantly higher, while energy expenditure significantly decreased, in food-restricted rats fed HF diet compared to controls. Mitochondrial efficiency and oxidative damage in skeletal muscle were significantly increased, while antioxidant defence was significantly lower in food-restricted rats fed HF diet, compared with controls. Finally, food-restricted rats fed HF diet exhibited significant reduction in subsarcolemmal mitochondrial mass. In conclusion, caloric restriction elicits higher mitochondrial efficiency and predisposes skeletal muscle to high fat-induced oxidative damage, which in turn could lead to impaired glucose homeostasis in food-restricted rats fed HF diet.

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## Correspondence

**S. Iossa**  
Department of Biology  
Complesso Universitario di  
Monte Sant'Angelo  
Edificio 7  
Via Cinthia  
80126 Napoli  
Italy  
Tel.: +39/81/2538 111  
Fax: +39/81/679 233  
[susioassa@unina.it](mailto:susioassa@unina.it)

## Introduction

It is well known that high-fat (HF) feeding in rodents can induce obesity and metabolic disorders that resemble the human metabolic syndrome. Accordingly, we have previously shown that HF feeding in adult rats elicited an increase in metabolic efficiency, thus leading to obesity development and insulin resistance [1,2]. Another physiological condition characterized by increased metabolic efficiency is the accelerated recovery of body fat that occurs during refeeding after caloric restriction [3]. Since this accelerated body fat recovery is evident in the absence of hyperphagia and during refeeding with low fat diet [4], it is conceivable that an enhanced efficiency for fat deposition is a fundamental physiological reaction to caloric restriction. In addition, studies of refeeding after caloric restriction in the rat have shown that the high metabolic efficiency that drives catch-up fat on a low-fat diet is exacerbated by refeeding on high-fat diets [5,6].

Changes in whole body metabolic efficiency reflect changes in single organs and tissues, most likely involving those which are major contributors to daily metabolic rate, such as liver and skeletal muscle [7]. It has been proposed that skeletal muscle is involved in the suppression of thermogenesis that underlies the high metabolic efficiency for accelerated body fat recovery after caloric restriction [8]. In addition, rats showing catch-up fat exhibit diminished subsarcolemmal (SS) mitochondrial mass and oxidative capacity in skeletal muscle [9], and altered mitochondrial energetic have been found in skeletal muscle after high fat feeding [1,2].

Taking into account that in Western society the diets are usually hyperlipidic and caloric restriction is often practiced by many people trying to lose body weight, we considered of interest to evaluate alterations in mitochondrial energetic in skeletal muscle elicited by short term high fat feeding in rats previously subjected to a caloric restriction regimen, in order to clarify the impact

of energy restriction on the metabolic response to high fat feeding. Since mitochondrial population is heterogeneous, composed of mitochondria located either beneath the sarcolemmal membrane (SS) or between the myofibrils (intermyofibrillar, IMF) [10] that exhibit different energetic characteristics and therefore can be differently affected by physiological stimuli [11], it is important that both these SS and IMF mitochondrial populations are separately studied.

After evaluating the impact of HF diet on whole body energy balance, we investigated the functionality of skeletal muscle SS and IMF mitochondrial compartments, by measuring oxidative capacity as well as efficiency of oxidative phosphorylation. Furthermore, oxidative damage and antioxidant defence in SS and IMF mitochondria were also determined.

## Materials and Methods

### Animals

Male Sprague-Dawley rats (Charles River, Italy) aged 6 weeks were adapted to room and cage environments for at least 1 week prior to the start of the experiment. The rats were caged singly in a temperature-controlled room ( $23 \pm 1^\circ\text{C}$ ) with a 12-h light/dark cycle, with free access to tap water and were maintained on a commercial nonpurified diet (4 RF21, Mucedola, Italy). Animals used in the present studies were maintained in accordance with Italian Health Ministry regulations and guidelines for the care and use of laboratory animals. All experimental procedures involving animals were approved by "Comitato Etico per la Sperimentazione Animale" of the University "Federico II" of Naples. Two groups of weight-matched animals were pair fed for a period of 1 week with HF diet (430 kJ metabolizable energy/day, corresponding to the spontaneous energy intake of rats with similar body weight and age). One group of rats (called R-HF, food-restricted rats refed HF diet) was food-restricted with non-purified diet for 2 weeks at approximately 50% of the spontaneous energy intake corresponding to 215 kJ metabolizable energy/day, and then fed with the HF diet, while the second group of rats was called C-HF (control rats fed HF diet) and was fed ad libitum for 2 weeks with nonpurified diet and then fed with the high-fat (HF) diet. The HF diet consisted, by energy, of protein 22.06, lipid 54.35, carbohydrate 23.59, and contained (g/kg): nonpurified diet 615, casein 121, methionine 1.3, choline 0.9, AIN vitamin mix 4.8, AIN mineral mix 17.1, sunflower oil 15.0, lard 225; metabolizable energy 17.90 kJ/g [estimated by computation using values (kJ/g) for energy content as follows: protein 16.74, lipid 37.66, and carbohydrate 16.74].

At the end of the 1 week period of isocaloric feeding on HF diets, the 2 groups of animals were killed by decapitation for measurements of body composition and energy balance, or for blood collection and skeletal muscle harvesting. At the start of the experimental period, some food-restricted rats as well as some control rats were killed by decapitation for measurements of initial body energy and lipid content.

### Body composition and energy balance

Carcasses were homogenized, and samples were analyzed for energy content by bomb calorimeter. Total body fat content was measured by the Folch extraction method [12]. Total body protein content was determined using a formula relating total energy value of the carcass, energy derived from fat, and energy derived from protein [5]; the caloric values for body fat and pro-

tein were taken as 39.2 and 23.5 kJ/g, respectively [13]. Energy balance measurements were conducted by the comparative carcass technique as detailed previously [14].

### Isolation of skeletal muscle mitochondria and assay of uncoupling protein

Hind leg muscles were rapidly removed and used for the preparation of homogenates and isolated SS and IMF mitochondria as previously reported [14]. Mitochondrial preparations were obtained by pooling skeletal muscle from 4 rats. Preliminary experiments have shown that contamination of SS and IMF mitochondria by other ATPase-containing membranes was lower than 10%, protease treatment of SS mitochondria had no effect on state 3 and 4 respiratory activities and addition of cytochrome c (3 nmol/mg protein) only enhanced state 3 respiration by approximately 25% and 10% in SS and IMF mitochondria, respectively. Determination of uncoupling protein 3 (UCP3) was carried out by Western blotting using UCP3 antibody (Chemicon International, CA, USA) as described previously [15].

### Measurements of mitochondrial respiration, uncoupling effect of fatty acids, and proton leak kinetics

Oxygen consumption was measured polarographically with a Clark-type electrode (Yellow Springs Instruments, OH, USA) at  $30^\circ\text{C}$ , using a medium containing 30 mmol/l KCl, 6 mmol/l  $\text{MgCl}_2$ , 75 mmol/l sucrose, 1 mmol/l EDTA, 20 mmol/l  $\text{KH}_2\text{PO}_4$  (pH 7.0), and 0.1% (w/v) BSA. In the presence of 0.6 mmol/l ADP, state 3 oxygen consumption was measured. State 4 was obtained in the absence of ADP. Respiratory substrates used were: succinate 10 mmol/l + rotenone 3.75  $\mu\text{mol/l}$ , glutamate 10 mmol/l + malate 2.5 mmol/l, palmitoylCoA 40  $\mu\text{mol/l}$  + carnitine 2 mmol/l + malate 2.5 mmol/l. Proton leak kinetics were obtained as previously reported [1]. Uncoupling effect of fatty acids was assessed by measuring the decrease in mitochondrial membrane potential as in [1], after the addition of 45  $\mu\text{M}$  or 65  $\mu\text{M}$  palmitate for SS and IMF mitochondria, respectively. These concentrations were selected to obtain a decrease in membrane potential, which is lower than that obtained in the transition from state 4 to state 3 condition (about 15–20 mV) in both SS and IMF mitochondria.

### Determination of mitochondrial mass, lipid peroxidation, aconitase, and superoxide dismutase (SOD) specific activity

Mitochondrial SS and IMF mass was assessed indirectly by 2 different approaches, namely (i) by measuring the activity of the mitochondrial marker enzyme citrate synthase (CS) in skeletal muscle homogenates and in isolated mitochondria, according to Srere [16], and (ii) by evaluating the mitochondrial yield. Lipid peroxidation was determined according to Fernandes et al. [17], by measuring thiobarbituric acid reactive substances. Mitochondria were solubilized in 1% Triton X-100, and active and total aconitase specific activity were measured according to Gardner [18] and Hausladen and Fridovich [19], respectively. SOD specific activity was measured according to Flohè and Otting [20].

### Blood parameters

The blood samples were centrifuged at  $1400 \times g_{av}$  for 8 min at  $4^\circ\text{C}$ . Plasma was removed and stored at  $-20^\circ\text{C}$ . Plasma insulin concentrations were measured using ELISA kits in a single assay to remove inter-assay variations (Mercodia AB, Uppsala, Sweden). Plasma glucose was measured by colorimetric enzymatic

**Table 1** Body composition, energy balance, plasma glucose, and insulin levels after 1 week of feeding on HF diet in control and food-restricted rats.

	C-HF	R-HF
Body weight (g)	264±2	262±5
Body energy (kJ/g)	9.0±0.1	9.6±0.2*
Body lipids (%)	14.4±0.1	15.6±0.1*
Body proteins (%)	14.5±0.1	14.3±0.2
Weight gain (g)	53±2	56±4
Fat gain (g)	20.0±0.8	28.8±1.7*
Protein gain (g)	6.1±0.2	8.5±1.8
Energy gain (kJ)	970±22	1282±56*
ME intake (kJ)	2865±79	2883±20
Energy expenditure (kJ)	1896±42	1601±44*
Gross energetic efficiency (%)	34±1	44±2*
Net energy expenditure (kJ)	1563±63	1180±64*
Net energy expenditure/ME intake (%)	58.6±1.5	40.9±2.9*
Insulin (ng/ml)	1.60±0.21	2.67±0.23*
Glucose (mg/dl)	235±8	231±11
HOMA index	21.5±3	35.1±4*

Values are the means±SEM of 6 different experiments

\* $p < 0.05$  compared to C-HF (unpaired, 2-tailed Student's *t*-test)

C-HF: Control rats fed HF diet; R-HF: Restricted rats fed HF diet

HOMA index =  $[(\text{Glucose (mg/dl)} \times \text{Insulin (mU/l)}) / 405]$

method using commercial kit (Pokler Italia, Genova, Italy). Insulin resistance was assessed by homeostasis model assessment (HOMA) index =  $[\text{Glucose (mg/dl)} \times \text{Insulin (mU/l)}] / 405$  [21]. All chemicals utilized were of analytical grade and were purchased from Sigma (St Louis, MO, USA).

### Statistical analysis

Data are provided as means±SEM. Statistical analyses were performed by 2-tailed, unpaired, Student's *t*-test or by nonlinear regression curve fitting. Probability values less than 0.05 were considered to indicate a significant difference. All analyses were performed using GraphPad Prism 4 (GraphPad Software, San Diego, CA, USA).

### Results

The data on body composition at the end of HF diet treatment are shown in **Table 1**. After 1 week of dietary treatment, the 2 groups of rats displayed similar body weight and body protein percentage, but food-restricted rats fed HF diet exhibited significantly greater fat percentage and body energy content than controls. In addition, plasma insulin concentrations, as well as the HOMA index, were significantly higher in food-restricted rats fed HF diet than in controls, while no differences were found in plasma glucose levels.

At the end of 1 week of diet treatment, food-restricted rats fed HF diet exhibited significantly greater gain in body lipid mass and energy than controls, while the gain in body protein and weight were not significantly different (**Table 1**). Energy balance data during diet treatment (**Table 1**) indicate that, notwithstanding similar energy intake between the 2 experimental groups, the greater gain in body fat and energy found in food-restricted rats fed HF diet resulted from lower values of energy expenditure and net energy expenditure (which can represent the cost of body energy maintenance). **Table 1** also shows a significant increase in gross energetic efficiency as well as a

decrease in net energy expenditure expressed as the percentage of ME intake in food-restricted rats fed HF diet than controls. Mitochondrial SS and IMF mass from skeletal muscle at the end of HF diet treatment were determined by using the mitochondrial marker enzyme CS. At the end of the diet treatment, CS activity/g tissue was found to be significantly lower in food-restricted rats fed HF diet than in controls, both in muscle homogenate (**Fig. 1b**) and in isolated SS mitochondria (**Fig. 1c**), whereas no differences were found in CS specific activity/mg protein in SS and IMF mitochondria (**Fig. 1e, f**). In addition, the yield of SS mitochondria from the food-restricted rats fed HF diet was lower than that from controls (**Fig. 1g**), whereas recovery of CS activity was unchanged in the food-restricted rats fed HF diet compared to controls (**Fig. 1a**).

Oxidative capacities of SS and IMF skeletal muscle mitochondria evaluated by using NAD, FAD and lipid substrate (**Table 2**) were found not to be affected by caloric restriction.

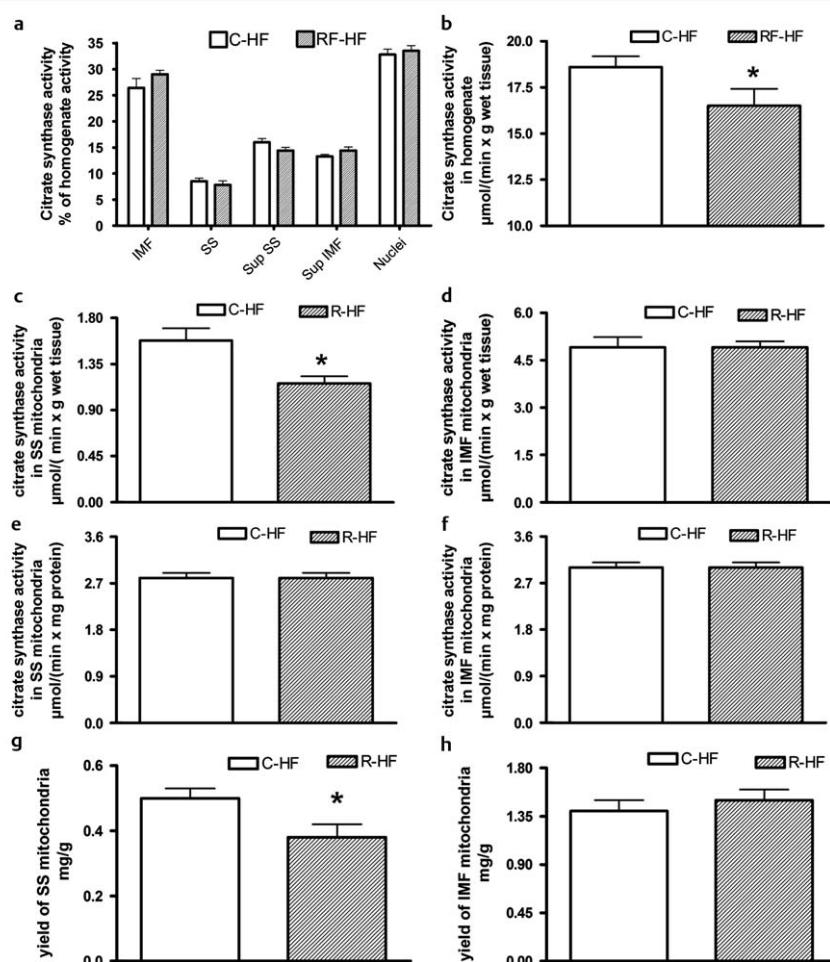
Mitochondrial energetic efficiency was assessed by determining mitochondrial proton leak and the uncoupling effect of fatty acid palmitate. The results on mitochondrial proton leak, assessed by titration of steady state respiration rate as a function of mitochondrial membrane potential in SS and IMF skeletal muscle mitochondria, are presented in **Fig. 2**. These titration curves are an indirect measurement of proton leak, since steady state oxygen consumption rate (i.e., proton efflux rate) in non-phosphorylating mitochondria is equivalent to proton influx rate due to proton leak. Comparisons of these curves, for SS and IMF mitochondria isolated from the 2 experimental groups at the end of the diet treatment, show that the proton leak was significantly lower in SS (**Fig. 2a**) and IMF (**Fig. 2b**) mitochondria from food restricted rats fed HF diet than in SS and IMF mitochondria from control rats. As for the uncoupling effect of palmitate, the decrease in mitochondrial state 4 membrane potential induced by palmitate was found to be significantly lower in IMF and SS mitochondria from food restricted rats fed HF diet than in IMF and SS mitochondria from control rats (**Fig. 2c**).

Lipid peroxidation and aconitase activity were measured and taken as indexes of cellular oxidative damage (**Table 3**). At the end of 1 week of diet treatment, food-restricted rats fed HF diet exhibited a significant decrease in active/total aconitase activity ratio compared to controls, while lipid peroxidation was significantly higher, indicating oxidative stress damage both in IMF and SS mitochondria. SOD specific activity, taken as an index of antioxidant defence, was found significantly lower in food-restricted rats fed HF diet, while UCP3 protein content, measured by Western blot analysis, was found not to be affected by caloric restriction (**Table 3**).

### Discussion

Evidence is presented here that feeding rats HF diet has a different impact on body energy balance, glucose homeostasis, mitochondrial energetics, and oxidative damage if the animals have been previously food-restricted. In fact, these rats exhibit diminished skeletal muscle mitochondrial mass, specifically in the SS mitochondrial compartment, proton leak and oxidative defence, together with an elevated HOMA index and hence impaired glucose homeostasis.

Firstly, our present results on energy balance and body composition indicate that acute exposure (1 week) to HF diet, if the rats have been previously food-restricted, elicits a significant



**Fig. 1** Percent recovery of citrate synthase activity **a**, citrate synthase activity/g wet tissue in skeletal muscle homogenate **b**, in SS **c**, and in IMF **d** mitochondria, citrate synthase activity/mg protein in SS **e** and in IMF **f** mitochondria, and yield of SS **g** and IMF **h** mitochondria after 1 week of feeding on HF diet in control and food-restricted rats. Values are the means  $\pm$  SEM of 6 different experiments. \* $p < 0.05$  compared to C-HF (unpaired, 2-tailed Student's *t*-test). C-HF: Control rats fed HF diet; R-HF: Restricted rats fed HF diet; SS: Subsarcolemmal mitochondria; IMF: Intermyo-fibrillar mitochondria; SupSS: Supernatant of subsarcolemmal mitochondria; SupIMF: Supernatant of intermyo-fibrillar mitochondria.

	Subsarcolemmal		Intermyofibrillar	
	C-HF	R-HF	C-HF	R-HF
<b>Glutamate</b>				
State 3	394 $\pm$ 19	411 $\pm$ 24	1017 $\pm$ 41	1006 $\pm$ 29
ngatoms oxygen/(min $\times$ mg protein)				
State 4	38.2 $\pm$ 1.1	35 $\pm$ 2.8	65.5 $\pm$ 3.0	63.2 $\pm$ 1.8
ngatoms oxygen/(min $\times$ mg protein)				
RCR	10.2 $\pm$ 0.8	11.7 $\pm$ 0.4	15.6 $\pm$ 0.7	16.0 $\pm$ 0.8
<b>Succinate</b>				
State 3	396 $\pm$ 9	409 $\pm$ 10	989 $\pm$ 15	1104 $\pm$ 3
ngatoms oxygen/(min $\times$ mg protein)				
State 4	89.3 $\pm$ 2.6	88.3 $\pm$ 1.1	238 $\pm$ 9	228 $\pm$ 4
ngatoms oxygen/(min $\times$ mg protein)				
RCR	4.4 $\pm$ 0.2	4.6 $\pm$ 0.1	4.3 $\pm$ 0.2	4.8 $\pm$ 0.3
<b>PalmitoylCoA</b>				
State 3	181 $\pm$ 8	192 $\pm$ 4	487 $\pm$ 15	507 $\pm$ 14
ngatoms oxygen/(min $\times$ mg protein)				
State 4	33.8 $\pm$ 1.9	30.8 $\pm$ 1.1	55.5 $\pm$ 1.5	51.5 $\pm$ 1.4
ngatoms oxygen/(min $\times$ mg protein)				
RCR	5.3 $\pm$ 0.7	6.2 $\pm$ 0.3	8.8 $\pm$ 0.6	9.9 $\pm$ 0.3

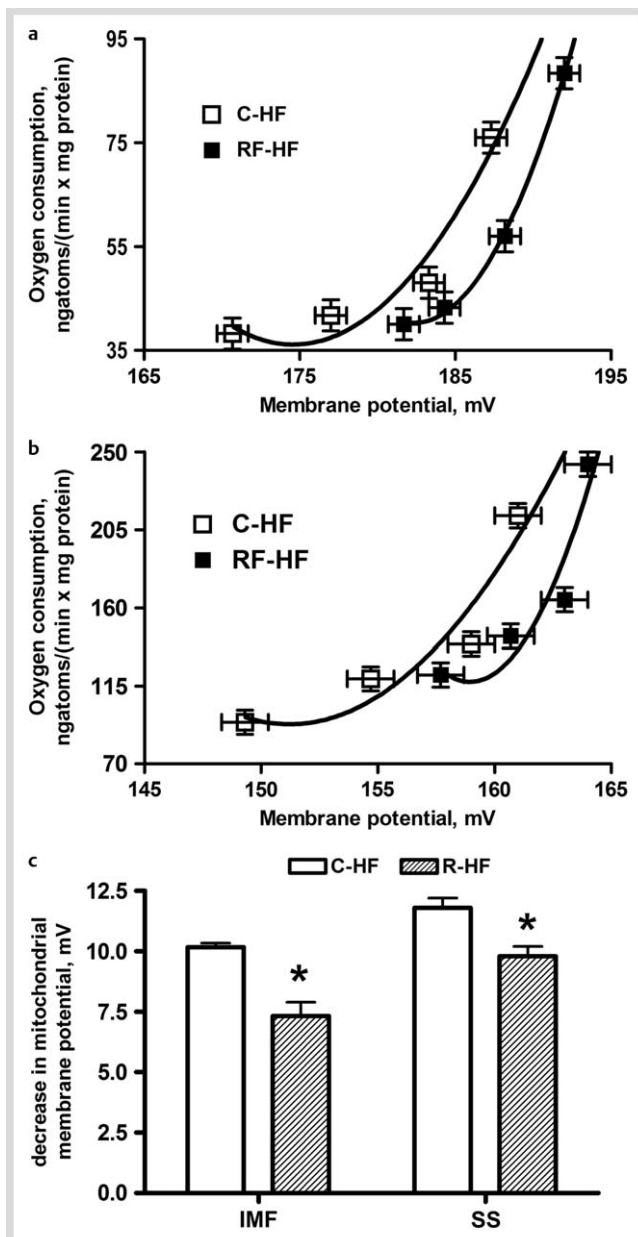
Values are the means  $\pm$  SEM of 6 different experiments

C-HF: Control rats fed HF diet; R-HF: restricted rats fed HF diet; RCR: respiratory control ratio

**Table 2** State 3 and 4 oxidative capacities of subsarcolemmal and intermyofibrillar skeletal muscle mitochondria after 1 week of feeding on HF diet in control and food-restricted rats.

increase in body energy as lipid. In fact, in these rats we found a decrease in energy expenditure that makes the energy balance more positive. It can be postulated that caloric restriction period as therapeutic slimming and HF feeding, although with different mechanisms, elicit a thrifty energy metabolism, whose effects

add to each other, and can be very dangerous in our modern society with a constant availability of diet rich in fat and, therefore, represent a major risk factor for obesity, type-2 diabetes, and cardiovascular diseases.



**Fig. 2** Kinetics of proton leak in SS **a** and IMF **b** mitochondria, and measurements of decrease in state 4 membrane potential after the addition of palmitate in SS and IMF mitochondria **c** after 1 week of feeding on HF diet in control and food-restricted rats. Values are the means  $\pm$  SEM of 6 different experiments. In **a** and **b**, nonlinear regression curve fits showed that proton leak was significantly ( $p < 0.05$ ) lower in IMF and SS mitochondria from restricted rats fed HF diet than the respective controls. In **c**, \* $p < 0.05$  compared to C-HF (unpaired, 2-tailed Student's *t*-test). C-HF: Control rats fed HF diet; R-HF: Restricted rats fed HF diet; SS: Subsarcolemmal mitochondria; IMF: Intermembranar mitochondria.

Since >90% of cellular energy is produced during oxidative phosphorylation in mitochondria, changes in mitochondrial mass (reflecting number, size or both), and/or efficiency could exert profound effects on energetic handling. To assess changes in mitochondrial protein mass, we utilized 2 approaches, namely (i) by measuring the activity of a mitochondrial marker enzyme CS, in skeletal muscle homogenates and in isolated SS and IMF mitochondria, (ii) by evaluating the mitochondrial yield (i.e., mg isolated protein/g starting wet tissue) in each mitochondrial subpopulation. Independently of these 2 approaches utilized,

we found that the mitochondrial mass was significantly reduced only in the SS compartment of food-restricted rats fed HF diet, thus indicating the previous caloric restriction period as the cause of changes in SS mitochondrial content in the skeletal muscle cells. In agreement, we found a similar decrease in SS mitochondria in rats at the end of caloric restriction [22].

Since an important factor that affects oxidative phosphorylation efficiency is the permeability of the inner mitochondrial membrane to  $H^+$  ions, we measured mitochondrial proton leak and uncoupling effect of fatty acids in isolated SS and IMF mitochondria. The results clearly indicate that caloric restriction increases oxidative phosphorylation efficiency in both mitochondrial populations, so that less fuel is oxidized to obtain the same amount of ATP. A decreased substrate burning by skeletal muscle is in agreement with the decrease in energy expenditure here found in food-restricted rats fed HF diet, given that skeletal muscle account for about 30% of whole-body energy expenditure [7]. Our present results are different from those previously obtained by us in food-restricted rats fed low fat diet [9], where we found a decreased mitochondrial efficiency in skeletal muscle, thus indicating that the effects of caloric restriction on energy metabolism in skeletal muscle depend on the fat content of the diet. Our data suggest that the above quantitative (in SS) and qualitative (in SS and IMF) variations in mitochondrial compartment could contribute to thrifty energy metabolism that favors recovery of fat during HF refeeding after caloric restriction.

The present data of lower proton leak and palmitate uncoupling effect are suggestive of increased mitochondrial reactive oxygen species (ROS) production. In fact, ROS production by the mitochondrial respiratory chain is higher when membrane potential increases [23, 24], so leading to an increased probability for electrons to react directly with dioxygen and to form superoxide and related ROS [25]. For these reasons, we also assessed the oxidative status of SS and IMF skeletal muscle mitochondria and in food-restricted rats fed HF diet we found signs of oxidative damage, both in the lipid component (in terms of lipid peroxidation) and in the protein component (in terms of damaged activity of aconitase, a very sensitive enzyme to ROS damage [17]). In agreement with oxidative damage in SS and IMF mitochondria from food-restricted rats fed HF diet, we found a decreased activity of SOD in SS and IMF mitochondria from these rats. Taking into account that impaired glucose homeostasis has been found only in food-restricted rats fed HF diet where SOD activity is lower, these results are consistent with the recent observation that overexpression of mitochondrial SOD in skeletal muscle from rats fed a HF diet ameliorated the reduction in muscle glucose uptake and that this effect was mediated through an altered redox state [26]. From our present results it appears that caloric restriction predisposes to oxidative stress when followed by HF feeding, in contrast with the postulated protective role of caloric restriction against oxidative damage [27]. It should be pointed out that there is also evidence in humans of a higher susceptibility to HF-induced oxidative stress after caloric restriction [28]. Thus, the extrapolation of our present findings from small rodents to humans suggests that in infants and children with faltered growth, as well as in adult humans after slimming therapy, nutritional rehabilitation with energy dense diet is dangerous in terms of oxidative stress, excessive adiposity and insulin resistance.

In conclusion, the present study shows that acute (only 1 week) HF feeding after a previous caloric restriction period is able to induce higher oxidative phosphorylation efficiency and

	Subsarcolemmal		Intermyofibrillar	
	C-HF	R-HF	C-HF	R-HF
Active aconitase (mU/mg protein)	58.8±5.6	57.0±5.4	77.1±4.3	63.2±3.0*
Total aconitase (mU/mg protein)	110.1±5.8	121.1±4.3	157.0±4.7	155.1±13.0
Active/Total aconitase	0.54±0.02	0.47±0.01*	0.49±0.02	0.41±0.02*
Superoxide dismutase (U/mg protein)	29.7±0.6	25.1±0.7*	35.3±1.2	24.0±0.2*
UCP3 protein content (arbitrary units/mg protein)	24.2±1.6	22.9±1.6	18.4±1.6	15.7±1.6
Lipid peroxidation (nmol of thiobarbituric acid reactive substances/mg protein)	2.4±0.2	3.1±0.2*	1.2±0.1	1.6±0.1*

Values are the means ± SEM of 6 different experiments. For UCP3 protein content values are the means ± SEM of 4 different experiments

\*p<0.05 compared to C-HF (unpaired, two-tailed Student's t-test)

C-HF: Control rats fed HF diet; R-HF: Restricted rats fed HF diet

**Table 3** Aconitase, superoxide dismutase specific activity, lipid peroxidation, and UCP3 protein content in intermyofibrillar and subsarcolemmal skeletal muscle mitochondria after 1 week of feeding on HF diet in control and food-restricted rats.

decreased oxidative defence in skeletal muscle mitochondria. As a consequence, caloric restriction predisposes to HF induced oxidative stress, which in turn could lead to impaired glucose homeostasis found in food-restricted rats fed HF diet. Therefore, therapeutic slimming followed by HF feeding in sedentary condition could have serious pathological implications.

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## Conflict of Interest

The authors declare that they have no conflicts of interest in the authorship or publication of this contribution.

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